16. (Amended) A method of restoring iodide transport to dedifferentiated thyroid cancer cells comprising the step of administering a demethylating agent in an amount effective to transcriptionally activate a thyroid specific therapeutic response element in a thyroid cancer cell that is defective in iodide transport, wherein said thyroid specific therapeutic response element is a sodium iodide symporter.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

REMARKS AND ARGUMENTS

Claims 1-19 are pending in this application. Applicant notes that by the Office Action of May 9, 2001, the Examiner has withdrawn claims 17-19 from further consideration.

Pursuant to the Examiner's request, Applicant affirms to prosecute the invention of Group 1, which was elected provisionally with traverse during a telephone conversation with Joseph Hyosuk Kim on March 29, 2001.

The following reply to the Office Action is in respect of the rejected claims 1-16.

Claims 1-5, 7-14 and 16 remain in this application. Claims 6 and 15 have been cancelled. Claims 1, 7, 8, 10, 12, 13, and 16 have been amended.

Claim rejections under 35 USC §112 first paragraph

Referring to page 3, item 10 of the Office Action, the Examiner rejected claims 1-15 for not being enabled by the specification. The present response includes amendments to the claims that the Applicant believes address the

concerns of the Examiner. In view of the amendments, the scope of the claims does not extend to a method for inducing the re-expression of any previously silenced gene encoding a therapeutic response element in a any cancerous cell, wherein any demethylating agent is administered to the cell. The amended claims are directed to a method for re-expressing a previously silenced thyroid gene in human cancerous thyroid cells by using a demethylating or differentiating agent. Support for the amended claims is found in Figures 4 and 6, page 15, line 29 to page 16, line 5, and in Table 2.

Referring to page 6 of the Office Action, the Examiner states that the deblocking agents (DMSFO, sodium butyrate, and phenylacetate) used by the inventor are not commonly known as demethylating agents, (lines 3-6). The Applicant recognizes this fact and had referred to these agents as putative demethylating agents (see page 2, lines 21-27; page 15, lines 29-34. In order to clarify this issue, Applicant has amended the claims to specify that the unblocking agent is a demethylating or a differentiating agent.

The specification discloses that agents phenylacetate, sodium butyrate and 5-azacytidine induce the re-expression of hNIS mRNA. Therefore, the specification is enabling with respect to the fact that administering an unblocking agent, which is a demethylating or a differentiating agent, results in re-expression of hNIS mRNA. The applicant respectfully disagrees with the Examiner's statement that "the specification provides no exemplification of the claimed method wherein a deblocking agent other than a demethylating agent is used." The Examiner's statement is contradictory to a prior statement on page 4 of the Office Action, wherein the Examiner admits that the specification "teaches that treatment of human thyroid papillary carcinoma cell lines....and the human benign follicular adenoma cell line... with 5-azacytidine, sodium butyrate, or phenylacetate restores the expression of the previously silenced (gene) encoding the human sodium/iodide symporter." As discussed above the claims have been amended to specify that the unblocking agent may be a demethylating or a differentiating agent.

The Examiner states that there are other mechanisms apart from a lack of gene expression, which account for the lack of I-uptake by a cancer cell, and cites a reference that describes the loss of I-uptake due to a mutation in the NIS gene (Matsuda et al). The cited reference relates to an issue that is not addressed by the present disclosure. The Examiner states that missense mutations affect NIS and iodine uptake, and therefore one cannot use the method of administering a putative demethylating agent to restore a gene whose defect encodes an inactive protein. The present invention however, relates to restoring expression of the hNIS gene in instances where the level of NIS mRNA is diminished. Demethylation of a gene that encodes an inactive protein due to an altered sequence would not restore protein function. A missense mutation that is related to a decrease in I-uptake need not necessarily correlate with a decrease in gene expression. In fact this is the case described in the reference cited by the Examiner. The reference describes a missense mutation in the NIS gene that relates to the inability to take up I, and wherein mRNA levels for NIS are 100 fold greater than in control cells. It also explains that the increase in mRNA levels may be a mechanism whereby the cell attempts to compensate for the protein's lack of function by overexpressing the gene. In this case the mRNA levels are not depressed, rather, they are overexpressed, and a person skilled in the art would not choose such a method to restore mRNA levels by demethylation. The present inventors have never asserted that they want to restore gene expression in cases where RNA is overexpressed.

The Examiner also states that given the toxicity of the deblocking agents, the invention cannot in good conscience be practiced without first performing extensive and undue experimentation". The specification teaches that administering the deblocking agents to thyroid cancerous cells, induces re-expression of the silenced NIS gene. The specification also teaches that the effects are attainable at concentrations between 0.5 and 1 mM sodium butyrate or phenyl acetate, and at concentrations between 0.5 and 1 uM azacytidine. With respect to azacytidine, the specification teaches that 1uM AzaC is toxic to KAT-10 cells, but that a 0.1 uM AzaC is not toxic to the same cells in which it

induces re-expression of the NIS gene. Therefore the specification establishes ranges of concentrations at which an effect is shown, and it would not require extensive experimentation to determine the optimal concentration within the given range at which the agent induces re-expression of the NIS gene without being toxic to the cell.

On page 7 of the Office Action, the Examiner states "that the specification does not exemplify the use of the claimed method, wherein the expression of a gene encoding an exogenous therapeutic response agent is restored.", and argues that the disclosure is not sufficient to enable one skilled in the art to make and/or use the claimed invention with a reasonable expectation of success without undue experimentation. On page 10 of the specification the reference of Shimura is disclosed, which describes the restoration of radioiodide uptake to "transformed rat thyroid cells, lacking endogenous NIS expression" by transfecting NIS cDNA into the transformed cells. Methods of transfecting genes into cells are well known, and form the basis of gene therapy. Shimura exemplifies a method for introducing the NIS gene into thyroid cells. Therefore, Applicant respectfully disagrees with the Examiner that the specification is not enabling.

Referring to item 12 on pages 10 and 11 of the Office Action, the Examiner rejected claims 1-16 as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Specifically the Examiner stated that "when given the broadest, reasonable interpretation, the claims encompass an extraordinarily large genus of genes, which encode a multitude of species of therapeutic response elements." The claims have been amended to encompass only thyroid-specific genes, and two sets of unblocking agents.

Referring to pages 13 and 14, item 14 of the Office Action, the Examiner rejected claims 1-16 for failing to point out and specifically claim the subject

matter which the applicant regards as the invention. Applicant has amended the claims, except for claim 11, to overcome the rejection based on indefiniteness, and in view of the amendment requests the Examiner to reconsider this rejection. Claim 11 was rejected for being indefinite because the claim recites the phrase "an untranslated region within the first exon", and that "the use of the phrase renders the claim indefinite because by definition an exon is translated..." Applicant respectfully disagrees with the Examiner that exons are translated. An exon is by definition a portion of a split gene that is included in the transcript of a gene and survives processing of the RNA in the cell nucleus to become part of a spliced messenger of a structural RNA in the cell cytoplasm. Exons generally occupy three distinct regions of genes that encode proteins. The first **which is not translated into protein**, signals the beginning of RNA transcription and contains sequences that direct the mRNA to the ribosomes for protein synthesis. The exons in the second region contain the information that is translated into the amino acid sequence of the protein. Exons in the third region are transcribed into the part of the mRNA that contains the signals for the termination of translation and for the addition of a polyadenylate tail. (R. C. King and W.S Stansfield in A dictionary of Genetics, Oxford University Press 1997, page 120). Therefore, not all exon sequences are translated into protein. Applicant believes it is unnecessary to amend the claim in question.

35 USC §102 Claim Rejections

Referring to page 15, item 16 of the Office Action dated May 9, 2001: the Examiner has rejected claims 1, 2, 4, and 14 under 35 USC §102(b) as being anticipated by Spath et al (Molecular and Cellular Biology 17: 1913-1922, 1997). The Examiner's reference will be referred to hereinafter as "Spath".
The Examiner asserted that Spath teaches a method for inducing re-expression of a previously silenced HNF1 gene in dedifferentiated liver cancer cells, said

method comprising administering to the cell an unblocking agent that re-activates expression of the gene (abstract).

For the Examiner's convenience, the following correspondence between Spath and (amended) claim 1 is provided:

Claim 1	**Spath**
(Amended) A method of expressing a	
[tumor] thyroid specific therapeutic	does not teach this element
response element in a human	does not teach this element
cancerous thyroid cell in which the response element was blocked from expression, comprising the step of administering an unblocking agent to the cancerous cell harboring a gene encoding the response element, thereby resulting in the expression of the response element,	
wherein the unblocking agent is a demethylating or a differentiating agent.	does not teach these elements

Spath teaches the re-expression of a non-specific transcription factor, HNF1, by transfecting liver cells with another transcription factor, HNF4. Spath also shows that treating the transfected liver cells with azacytidine did not further increase the expression of HNF1, and more importantly, azacytidine did not result in the re-expression of endogenous HNF4.

Spath does not teach either a method for re-expressing thyroid specific therapeutic elements, nor using the deblocking agents claimed in 1. In order to be an anticipation of a claim, a reference must teach each and every element of the claim, including the relationship between the elements. If any element is not fully taught by the reference, the rejection cannot be sustained. Spath does not

teach each element of claim1. It follows that a rejection of claim 1 under 35 USC §102 (b) is inappropriate.

As claims 2, 4, and 14 depend on claim 1, it follows that Spath cannot teach each element of claims 2, 4, and 14, and a rejection of claims 2, 4, and 14 under 35 USC §102(b) is also inappropriate.

Referring to page 16, item 17 of the Office Action dated May 9, 2001, the Examiner has rejected claims 1-4, and 14, under 35 USC §102(b) as being anticipated by Avvedimento et al (Cell 58: 1135-1142, 1989). The Examiner's reference will be referred to hereinafter as "Avvedimento".
The Examiner asserted that Avvedimento "teaches a method for activating the re-expression of a gene encoding the therapeutic response element, thyroglobulin in cancer cells derived from the thyroid by administering an unblocking agent, namely 5-azacytidine, to the cells (abstract)".

For the Examiner's convenience, the following correspondence between Avvedimento and (amended) claim1 is provided:

Claim 1	**Avvedimento**
(Amended) A method of expressing a [tumor] thyroid specific therapeutic response element in a human cancerous thyroid cell in which the response element was blocked from expression, comprising the step of administering an unblocking agent to the cancerous cell harboring a gene encoding the response element, thereby resulting in the expression of the response element, wherein the unblocking agent is a demethylating or a differentiating agent.	does not teach this element

Avvedimento teaches using AzaC to re-activate a thyroglobulin promoter that drives the expression of G418 in rat thyroid cells that were transformed with a Kirsten murine sarcoma virus. These cells are clearly different from that of the cells of the present invention.

In order to be an anticipation of a claim, a reference must teach each and every element of the claim, including the relationship between the elements. If any element is not fully taught by the reference, the rejection cannot be sustained. Avvedimento does not teach each element of claim 1. It follows that a rejection of claim 1 under 35 USC §102 (b) is inappropriate.

As claims 2-4, and 14, depend on claim1, it follows that Avvedimento cannot teach each element of claims, and a rejection of claims 2-4, and 14 under 35 USC §102(b) is also inappropriate.

Referring to items 18 and 19 on pages 17 and 18 of the Office Action dated May 9, 2001, the Examiner has rejected claims 1, 2, 4, 12, and 13 under 35 USC §102(b) as being anticipated by Schmutzler et al (Biochemical Biophysical Research Communications 240:832-838, 1997). The Examiner also rejected claims 1, 2, 4, 12, 13, and 16 under 35 USC §102(b) as being anticipated by Van Herle (Journal of Clinical Endocrinology and Metabolism 71: 755-763, 1990). The Examiner's references will be referred to hereinafter as "Schmutzler" and "Van Herle".

Considering the rejections of claims 1, 2, 4, 12, and 13 based on Schmutzler and Van Herle, the Examiner asserted that Schmutzler teaches a method for inducing the expression of a previously silenced gene encoding the human sodium iodide symporter (hNIS), a therapeutic response element, in dedifferentiated thyroid cancer cells by administering retinoic acid to the cells (abstract). The Examiner also asserted that Van Herle teaches a method for restoring the iodide transport to dedifferentiated thyroid cancer cells, namely follicular carcinoma, said method comprising a step of administering a demethylating agent, namely retinoic acid..."

For the Examiner's convenience the following correspondence between Schmutzler, Van Herle and (amended) claim1, is provided:

CLAIM 1	Schmutzler	Van Herle
(Amended) A method of expressing a [tumor] thyroid specific therapeutic response element in a human cancerous thyroid cell in which the response element was blocked from expression, comprising the step of administering an unblocking agent to the cancerous cell harboring a gene encoding the response element, thereby resulting in the expression of the response element, wherein the unblocking agent is a demethylating or a differentiating agent.	teaches away	does not teach this method

Schmutzler teaches that retinoic acid, increases NIS mRNA levels in follicular thyroid carcinoma cell lines FTC-133 and FTC-238 (page 833, column 2). However, Schmutzler teaches away from using retinoic acid to induce iodide transport, because RA did not stimulate iodide transport in any of the cell lines that had been treated with RA (page 834 column 2). Therefore Schmutzler cannot anticipate claim 1 under 35 USC §102.

Van Herle teaches the inhibition of thyroid cell tumor growth by 13 *ois*-RA as measured by dose-dependent [^{3}H]- thymidine incorporation. Van Herle also teaches that 13 *cis*-RA increases the ^{131}I uptake of the tumor cells by four-fold above baseline. However, Van Herle does not relate the gain of iodide transport to the re-expression of a thyroid specific response element. Van Herle, neither teaches the methylation status of the NIS gene, nor does he determine the effect of an unblocking agent on the level of NIS mRNA. Therefore, claim 1 cannot be anticipated by Van Herle under 35 USC 102.

As claims 2, 4, 12, and 13 depend on claim 1, it follows that neither Schmutzler nor Van Herle cannot teach each element of claims, and a rejection of claims 2, 4, 12, 13, and 15 under 35 USC §102(b) is also inappropriate.

For the Examiner's convenience, the following correspondence between Van Herle and (amended) claim 16, is provided:

Claim 16	**Van Herle**
(Amended) A method of restoring iodide transport to dedifferentiated thyroid cancer cells comprising the step of administering a demethylating agent in an amount effective to transcriptionally activate a thyroid [tumor] specific therapeutic response element in a thyroid cancer cell that is defective in iodide transport, wherein said thyroid [tumor] specific therapeutic response element is [the] a sodium iodide symporter.	does not teach this element does not teach this limitation

Van Herle teaches that a concentration of up to 10 μM retinoic acid reduces the proliferation, and it increases the uptake of ^{131}I by four-fold in thyroid follicular cell line when compared to normal cells. Van Herle does not teach using a demethylating agent to activate the transcription of a thyroid specific response element. Therefore, Van Herle neither teaches nor suggests all the elements of claim 16. It follows that a rejection of claim 16 under 35 USC §102 (b) is inappropriate.

Referring to page 19, item 20 of the Office Action, the Examiner has rejected claims 1, 2, 5-8, and 11 under 35 USC §102(b) as being anticipated by

Swafford et al (Molecular and Cellular Biology 17: 1366-1374,1997). The Examiner's references will be referred to hereinafter as "Swafford".

The Examiner asserted that Swafford teaches a method for "restoring the expression of a silenced gene encoding a therapeutic response element, namely p16INK4a by administering to a cancer cell an unblocking agent, namely 2'-deoxy-5-azacytidine (abstract).

For the Examiner's convenience, the following correspondence between Swafford and (amended) claim 1 is provided:

CLAIM 1	**Swafford**
(Amended) A method of expressing a [tumor] thyroid specific therapeutic response element in a human cancerous thyroid cell in which the response element was blocked from expression, comprising the step of administering an unblocking agent to the cancerous cell harboring a gene encoding the response element, thereby resulting in the expression of the response element, wherein the unblocking agent is a demethylating or a differentiating agent.	does not teach this element does not teach this element

Swafford teaches using the inhibitor of DNA methyltransferase, 2-deoxy-5-azacytidine to re-express the gene p16INK4a in rat non-small-cell lung cancers. Swafford does not teach a method for re-expressing genes in cancerous thyroid cells, nor does he teach the unblocking agents of the present application.
In order to be an anticipation of a claim, a reference must teach each and every element of the claim, including the relationship between the elements. If any element is not fully taught by the reference, the rejection cannot be sustained.

Swafford does not teach each element of claim 1. It follows that a rejection of claim 1 under 35 USC §102 (b) is inappropriate.
As claims 2, 5,7, 8 and 11 depend on claim1, it follows that Swafford cannot teach each element of claims, and a rejection of the dependent claims under 35 USC §102(b) is also inappropriate.

35 USC §103 Claim Rejections

Referring to page 20, item 22 of the Office Action of May 9, 2001, the Examiner rejected claims 1, 2, 3, and 5-11 as being unpatentable under 35 USC § 103(a) over Swafford et al (Molecular and Cellular Biology 17: 1366-1374, 1997, hereinafter Swafford).

The Examiner asserted that "it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to substitute the unblocking agent of Swafford...", 2-deoxy-5-azacytidine for DMFO or 5-azacytidine. As stated above, Swafford teaches restoring the expression of the p16 gene in non-small-cell lung cancers. Swafford neither teaches nor suggests the method claimed in the present application.

In order to form a proper obviousness rejection of a claim under 35 USC §103(a), the reference must teach or suggest each element of the claim, including the relationship between the elements. If any element is not fully taught by the reference, the rejection cannot be sustained. As discussed above, Swafford does not teach or suggest all the elements and limitations of claim 1. Therefore, the rejection of claim 1 under 35 USC §103(a) does not apply.
As claims 2, 3, 5, 7, 8, 10, and 11 depend on claim 1, it follows that Swafford cannot teach each element of the claims, and a rejection under 35 USC § 103(a) is also inappropriate.

Referring to page 22. item 23 of the Office Action, the Examiner rejected claims 1, 2, 3-11, and 14 as being unpatentable over Graff et al (Cancer Research 58: 2063-2066, 1998).

The Examiner asserted that Graff teaches that the CpG islands contained in the regulatory element and coding regions of E-cadherin are densely methylated, that thyroid carcinoma cells dedifferentiate "since the expression of E-cadherin is lost"; and that "the dense hypermethylation of the CpG islands ... of the gene encoding E-cadherin in dedifferentiated thyroid cancer cells causes the expression of the gene to be lost." "It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to treat the dedifferentiated thyroid cancer cells of Graff et al with a demethylating agent, namely 5-azacytidine or difluoromethylornithine, to cause the demethylation of the gene encoding the therapeutic element E-cadherin..." "Finally, it would have been prima facie obvious ... to use a demethylating agent, namely 5-azacytidine or difluoromethylornithine, to induce re-expression of an exogenous gene encoding a therapeutic response element in a thyroid cancer cell ...".

For the Examiner's convenience, the following correspondence between Graff and (amended) claim 1, is provided:

CLAIM 1	**Graff**
(Amended) A method of expressing a [tumor] thyroid specific therapeutic response element in a human cancerous thyroid cell in which the response element was blocked from expression, comprising the step of administering an unblocking agent to the cancerous cell harboring a gene encoding the response element, thereby resulting in the expression of the response element,	does not teach this element does not teach this element

wherein the unblocking agent is a demethylating or a differentiating agent.	does not teach this element

Applicant agrees with the Examiner that "Graff et al does not teach [that] a method for inducing the re-expression of the previously silenced gene encoding E-cadherin in the dedifferentiated thyroid cancer cells by administering to the cells a demethylating agent, namely 5-azacytidine or difluoromethylornithine". Graff teaches aberrant methylation of E-cadherin 5' CpG island in human thyroid cancer cell lines. Furthermore, Graff does not teach either a method for re-expressing therapeutic response elements, thyroid tumor specific therapeutic response elements or any agents that need to be used to perform the method.

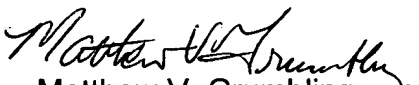
In order to form a proper obviousness rejection of a claim under 35 USC §103(a), the reference must teach or suggest each element of the claim, including the relationship between the elements. If any element is not fully taught by the reference, the rejection cannot be sustained. As shown in the table above, Graff does not teach or suggest all the elements and limitations of claim 1. Therefore, the rejection of claim 1 under 35 USC §103(a) does not apply. As claims 2-5, 7, 8, 10, 11, and 15 depend on claim 1, it follows that Graff cannot teach each element of the claims, and a rejection under 35 USC § 103(a) is also inappropriate.

For the foregoing reasons, Applicants submit that the present application is in condition for allowance. Applicants therefore respectfully request timely issuance of a Notice of Allowance.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

MCDERMOTT, WILL & EMERY



Matthew V. Grumbling
Registration No. 44,427

600 13th Street, N.W.
Washington, DC 20005-3096
(202) 756-8000 MVG:EQ:amz
Date: September 5, 2001
Facsimile: (202) 756-8087

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at line 29 of page 15 has been amended as follows:

-- Seven human thyroid neoplastic cell lines, devoid of hNIS mRNA expression under basal monolayer conditions, were treated with putative chemical demethylation agents in an attempt to restore hNIS expression. These cell lines were derived from three papillary carcinomas (NPA'87, KAT-5, and KAT-10), two follicular carcinomas (WRO82 and MR087) and two benign follicular neoplasms (KAK-1 and [KAK-10] KAK 50) (5). Three different demethylation or redifferentiation agents (*viz.*, sodium butyrate, phenylacetate and 5-azacytidine) were tested on each of 7 cell lines for their ability to induce re-expression of hNIS mRNA. Re-expression of hNIS mRNA was achieved in all three of the papillary cell lines and one of the benign follicular adenomas under at least one treatment condition (Table 2). Figure 4, a and b, demonstrate the hNIS mRNA re-expression in cell lines KAK-1 and NPA'87, respectively.

IN THE CLAIMS:

Claims 6 and15 have been cancelled.

Claims 1, 7, 8, 10, 12, 13 and 16 have been amended as follows:

1. (Amended) A method of expressing a thyroid [tumor] specific therapeutic response element in a human cancerous thyroid cell in which the response element was blocked from expression, comprising the step of administering an

unblocking agent to the cancerous cell harboring a gene encoding the response element, thereby resulting in the expression of the response element [.],
and wherein the unblocking agent is a demethylating or a differentiating agent.

7. (Amended) The method according to claim 5, wherein said [demethylating] unblocking agent is dimethylsulfoxide, sodium butyrate, phenylacetate, or 5-azacytidine.

8. (Amended) The method according to claim 5, wherein said [demethylating] unblocking agent is a compound that inhibits DNA-methyltransferase activity.

10. (Amended) The method of claim [9] 1, wherein said unblocking agent is difluoromethylornithine [DMFO, and] or adenosyl-1,8-diamino-3-thio-octane.

12. (Amended) The method according to claim 1, wherein said response element is a sodium-iodide symporter [NIS].

13. (Amended) The method according to claim 12, wherein said response element is a human sodium-iodide symporter [hNIS].

16. (Amended) A method of restoring iodide transport to dedifferentiated thyroid cancer cells comprising the step of administering a demethylating agent in an amount effective to transcriptionally activate a thyroid [tumor] specific therapeutic response element in a thyroid cancer cell that is defective in iodide transport, wherein said thyroid [tumor] specific therapeutic response element is [the] a sodium iodide symporter.